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PATENT COOPERATION TREATY

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From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

22330 WO - BUR

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/012461	04.11.2004	04.11.2003

International Patent Classification (IPC) or both national classification and IPC
G01N33/574, C12Q1/68

Applicant
ROCHE DIAGNOSTICS GMBH

Termin 1

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

Termin 2
04.09.2005
(24.07.05)

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-27 (partially)

because:

☒ the said international application, or the said claims Nos. 22-27 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 1-27 (partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-27 (partially)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18,20,21
	No:	Claims	19
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 26 and 27 pertain to a reference data bank for distinguishing AML subtype AML_inv(3) from other AML subtypes including 11q23, inv(16), AML_normal, t(15;17), and t(8;21).
A data bank as such is characterised only by data contained in said data bank, which are considered to be a mere presentation of information. No international preliminary examination is carried out for the subject-matter of said claims pursuing the provisions of Rule 67.1(v) PCT.
It should further be noted that the technical information presented under points (a) and (b) of claim 26 is related to the method of constructing said data bank and is therefore no characterising technical feature of the data bank as such, claimed in claim 26.
An analogous argumentation also applies to the subject-matter of claims 22-25.
Presentation of information is not patentable whether the claims are directed to the presentation of the information per so or to apparatus for presenting the information which are solely defined by the information recorded (see also the Preliminary Examination Guidelines, Chapter 9, Item 9.12). Again, the method for obtaining a data bank does not define the data bank as such.

Re Item IV

Lack of unity of invention

1. The application lacks unity within the meaning of Rule 13.1 PCT.
   The problem to be solved in the present application is the provision of markers for distinguishing AML subtype AML_inv(3) from other AML subtypes including 11q23, inv(16), AML_normal, t(15;17), and t(8;21), and for distinguishing said subgroups from each other.
   The single general concept which can be identified a priori as linking the various inventions and which forms a solution to the above problem relates to the use of

"markers for AML subtypes". The use of marker genes/nucleotides disclosed in tables 1-4 form 1800 different solutions to the above problem.
However, the concept of using marker genes for distinguishing different leukemia subtypes is know in the art.

The document WO-A-03/039443 (**D1**) describes novel genetic markers for leukemias, identified using differential gene expression analysis on Affymetrix GeneChips. In the table on page 836, the markers ARHH and STAM are disclosed for discriminating AML_MLL/t(11q23) (see also page 114, line 3 in Example 7) against all other AML subtypes. Said markers are included in table 1.1 of the present application for the same purpose.
In the table on page 809, the markers CTSW, CALR, STAB1, and MPO are disclosed for discriminating AML_t(15;17) from all other subtypes. Said markers are included in table 1.5 of the present application for the same purpose.
In the table on page 835, the markers SERPINF1, DRT18, ARHGAP10, KCNK17, CRA, and TNFSF13 are disclosed for discriminating AML_inv(16) from AML_t(8;21). Said markers are included in table 2.9 of the present application for the same purpose.

The document Schoch et al., PNAS (2002) Vol. 99(15), pp. 10008-10013 (**D2**), describes a method for distinguishing several forms of AML based on their gene expression profile as determined by using an Affymetrix GeneChip. Class prediction is performed using weighted voting. In tables 1 and 2, sets of genes are disclosed which are sufficient to distinguish between different leukemia subtypes. One of the markers of table 1, AF013570 (=MYH11), which is used to differentiate t(15;17) vs. inv(16) is described for the same purpose in table 2.8 of the present application. M26326(=KRT18) and AF013611 (=CTSW) of table 1 of D2 which are used to differentiate t(15;17) vs. t(8;21) are described for the same purpose in table 2.15 of the present application.
CLECSF2 of table 1 of D1 is used to differentiate t(15;17) vs. the rest and is included for the same purpose in table 1.5 of the present application.

Kohlmann et al., Genes, Chromosomes & Cancer (2003), Vol. 37, pp. 396-405 (**D3**) discloses molecular characterisation of acute leukemias by use of microarray

technology.
Several specific markers for distinguishing AML subtypes are identified.
In table 2, ARHGAP4 is disclosed for distinguishing t(11q23)/MLL from t(15;17). This marker is included for the same purpose in table 2.4 of the present application.
HLA-DPA1 is discloses for distinguishing AML_inv(16) from AML_t(15;17). This marker is included in table 2.8 of the present application for the same purpose.

The document Sood et al. (1999) Leukemia, Vol. 13, pp. 348-357 (**D4**) discloses expression of a specific transcription repressor EVI1 as a result of inv(3) chromosomal rearrangement (see abstract).

The document Kohlmann et al. (2002) Blood, Vol. 100(11), Abstract No. 308 (**D5**) discloses marker for distinguishing t(11q23)/MLL positive ALL and AML.

In the light of D1-D5, each document taken alone, the above identified single general concept is not novel and inventive and thus cannot be the single general inventive concept as required by Rule 13.1 PCT.
The present invention is thus considered not the fulfil the requirements of unity as laid down in Rule 13.1 PCT.

No other technical features could be identified that form a technical relationship among each of the separate inventions claimed and which could be considered as same or corresponding special technical features within the meaning of Rule 13.2 PCT.

The first invention was searched, namely methods relating to distinguishing AML subtypes, in particular AML t(11q23) from other subtypes using HOXB2 as a marker; kits and apparatus for distinguishing AML t(11q23) from other subtypes using said marker.

2. The Examining Authority considers that the following separate inventions or groups of inventions are not so linked as to form a single general inventive concept:

Invention 1: **Claims 1-27 (all partially)**

A method for distinguishing AML subtypes, in particular AML t(11q23) from other subtypes, the method comprising determining the expression level of the marker HOXB2. Use of said marker for the manufacture of a diagnostic. A diagnostic kit containing said marker and an apparatus comprising a reference data bank, wherein the reference data bank is obtainable by determining the expression level of HOXB2.

Inventions 2-1800: Claims 1-27 (all partially)

Methods for distinguishing AML subtype AML_inv(3) from other AML subtypes including 11q23, inv(16), AML_normal, t(15;17), and t(8;21), and for distinguishing said subtypes from each other, the method comprising determining individually the expression level of the markers listed in tables 1.1, positions 2-50, tables 1.2-1.6 and in tables 2-4. Use of said markers for the manufacture of diagnostics. Diagnostic kits containing said markers and apparatus comprising a reference data bank, wherein the reference data bank is obtainable by determining the expression levels of said markers.

The following assessment of novelty and inventive step will only pertain to subject-matter for which a search report has been established, i.e. invention 1.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.1 Claim 19 does not meet the requirements of Article 33(2) PCT.
Claim 19 refers to kit for distinguishing leukemia subtypes containing at least HOXB2. This must be construed as meaning merely a reagent suitable for carrying out the method. The intended use of a product is not a technical feature of the product per se. Therefore, commercially available microarrays, such as the U133 microarrays of Affymetrix, comprising HOXB2 specific probes are novelty-destroying for the subject-matter of claim 19 within the meaning of Article 33(2) PCT.

1.2 Claims 1-18 and 20-21 are novel within the meaning of Article 33(2) PCT, since the

prior art does not teach the use of HOXB2 as a marker for distinguishing AML_t(11q23) from all other subtypes, or kits and apparatus comprising a reference for leukemia subtypes based on HOXB2 expression.

2.1 Claim 1 does not meet the requirements of Article 33(3) PCT.
Documents D1-D3, each of which could be considered to represent the most relevant state of the art, disclose markers for distinguishing leukemia subtypes such as t(15;17), inv(16), t(8;21) and 11q23/MLL.
The underlying objective technical problem may therefore be seen in providing a further marker for distinguishing AML subtypes.
As already pointed out under item IV,1. above, the use of differential gene expression analysis using microarrays of gene probes for defining leukemia subtypes is described in detail in documents D1-D3. In addition, several other documents pertain to the concept of identifying gene expression profiles in order to characterise leukemia subtypes (see for example Schoch et al. (2002) Blood, Vol. 100(11) Abstract No. 1204 (**D6**); Schoch et al. (2001) Blood, Vol. 98(11 Part 1), pp. 92a-93a (**D7**); EP-A-1 308 522 (**D8**)) .
Moreover, methods for classifying samples based on gene expression data have become common general knowledge in the art, also in the field of leukemia diagnosis (see for example EP-A-1 043 676 (**D9**), the whole document; Kohlmann et al. (2002) Blood, Vol. 100(11), Abstract No. 4287 (**D10**)).
The above referred-to documents represent a non-exhaustive list of documents dealing with the identification of marker genes indicative of a specific leukemia subtype.

In particular documents D1-D4 and D6-D7 contain direct pointers that it is possible to identify gene markers which are specific for a certain AML subtype and thus enable an unambiguous identification of said subtypes. Document D3 specifically addresses the problem of distinguishing AML_(11q23) for other subtypes (see item IV.1).

Moreover, the use of HOXB2 as a marker does not appear to be associated with an unexpected and surprising technical effect in view of the above-cited documents, and in particular document D3, which could confer an inventive step compared to other markers identified by gene expression profiling using standard microarray technology.

It would therefore be obvious for a person skilled in the art to use differential gene expression based on microarray analysis in order to identify further markers, e.g. HOXB2, for specific leukemia subtypes in view of state of the art as exemplified in documents D1-D10 in order to solve the above-stated problem.

Hence, claim 1 cannot be considered as being inventive within the meaning of Article 33(3) PCT.

2.2 Claims 2-18 and 20-21 refer to standard embodiments in the art of microarray analysis and diagnostics and do not add technical features which would confer an inventive activity.
Claims 2-18 and 20-21 thus do also not meet the requirements of Article 33(3) PCT.

3. Should the objection under Rule 67.1(v) be overcome, the applicant is referred to documents Dugas et al. (2002), In silico biology, Vol. 2, pp. 383-391 (**D11**) and Dugas et al. (2001) Leukemia, Vol. 15, pp. 1805-1810 (**D12**), which disclose databases containing data from patients suffering from leukemia. Said data include characterisation of subtypes, and correlation of cytogenetic findings with, e.g., microarray data (D12: page 1807, col. 2; D11: the whole document). Therefore, claims pertaining to the generation of reference databases for the analysis of leukemia subtypes based on gene expression data could not be considered as being novel (Article 33(2) PCT).

Re Item VIII
Certain observations on the international application

1. In order to avoid any unclarity within the meaning of Article 6 PCT, abbreviations should be defined the first time they are mentioned in the claims.

2. Notwithstanding the objection of lack of unity raised under item 2. above, claim 1 does not meet the requirements of Article 6 PCT. The excessive use of "and/or" for defining various possible embodiments in claim 1 as well as the introduction of an

enormous number of possible marker combinations through the use of the term "at least one polynucleotide" in each of said possible embodiments, the claim lacks conciseness, contrary to the requirements of Article 6 PCT.